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Full Paper

Electrochemical Determination of Metronidazole using Nanostructured Over-Oxidized Polypyrrole

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Abstract- Pencil graphite electrodes (PGE) were modified with over-oxidized nanofiber structured polypyrrole (PPyNF-Ox) by electropolymerization method. The performances of the pencil graphite electrodes modified with overoxidized nanofiber structured polypyrrole (PGE/PPyNF-Ox) were investigated for metronidazole (MNZ) by the differential pulse voltammetry (DPV) technique. The electrochemical signals of nanofiber and non-nanofiber electrodes for metronidazole were also determined. It was determined that over-oxidation increased the reduction current of MNZ. Surface morphology of the modified electrodes was determined by scanning electron microscopy (SEM) and electrochemical characterization was determined by cyclic voltammetry (CV). The reduction current of metronidazole was found to be diffusion-controlled. Electrochemical data showed that MNZ reduction occurred with the transfer of 4 electrons and 4 protons. The PGE/PPyNF-Ox exhibited a linear response to MNZ in the 5-500 µM concentration range. The limit of detection for PGE/PPyNF-Ox in MNZ determination was calculated as 3.8 µM and the limit of quantification was calculated as 12.8 µM. Interferent species did not significantly affect the results in metronidazole determination. The electrochemical signal obtained with the modified electrodes did not change for 20 days. In addition, the performance of these modified electrodes in real samples was tested for drug tablets containing MNZ. The optimum working pH for PGE/PPyNF-Ox is 8.0 and therefore, it offers the opportunity to work in conditions close to the pH value of body fluids.

Keywords- Conducting polymer; Modified pencil graphite electrode; Metronidazole sensor; Over-oxidation

1. INTRODUCTION

Metronidazole (MNZ) is commonly administered in poultry to treat bacterial and protozoal infections owing to its strong bactericidal effects, efficient oral absorption, lack of cross-resistance, high bioavailability, and inhibitory properties. MNZ is also recognized for its effectiveness against anaerobic bacteria and parasitic antigens [1]. MNZ is also used to treat human diseases such as brain, bone, and blood infections [2]. Since MNZ causes weight gain in animals, it is used by adding it to poultry and fish feeds. It causes accumulation in wastewater, especially from meat industries and fish farms. These antibiotics have been detected in sewage treatment plants at mg L^{-1} concentration levels [3]. Therefore, developing an effective and accurate method for determining MNZ is extremely necessary from a medical and environmental perspective.

Many biologically important substances, such as MNZ, are determined using electrochemical methods. Electrochemical methods provide advantages such as easy use, low cost, and short determination time. Electrochemical methods, due to their portability, easy use, and good reproducibility, are increasingly favoured for determining electroactive species, including biologically essential substances like MNZ [4]. These advantages of electrochemical methods depend on the performance of the working electrodes. Modifying the working electrode surface with different molecules can enhance sensitivity, reduce the voltage required for electrochemical reactions, and improve selectivity [5]. In addition, working electrodes modified with molecules that have a catalytic effect enable higher current to be obtained at lower voltages [6]. MNZ glassy carbon has an aromatic nitro group that can easily undergo reduction reactions in various electrode systems such as gold, carbon paste, and carbon fiber electrodes. Analytical performance may decrease due to surface contamination and obstruction of redox reactions, especially when bare electrodes are used. Thanks to the use of modified electrodes, electrode contamination is reduced as the sensitivity and selectivity of the electrochemical response are improved [4].

Conductive polymers are preferred as electrode materials due to their appropriate chemical, electronic, and mechanical properties in modifying electrochemical sensors [7]. Conducting polymers, which can be synthesized with economical, fast, and simple methods, have many uses such as diode production, rechargeable batteries, corrosion protection, transistors, and biosensors [8]. Polypyrrole (PPy), one of the widely used conductive polymers, is chemically and mechanically durable, can be easily synthesized electrochemically on different materials even in neutral environments, and has biological compatibility, making polypyrrole preferred in many studies [9]. On the other hand, PPy has poor properties such as brittleness and low surface area. Therefore, the way to improve structural and physical properties is to develop different composite materials containing PPy or nanostructured PPy. Another method to enhance these properties is by depositing PPy onto a carbon-based material. Recent research has shown that nanocomposite materials obtained by combining PPy and carbon have better conductivity values than PPy [10,11].

Given the advantages of electrochemical methods and modified electrodes, it would be beneficial to use them for MNZ determination. A literature review reveals numerous studies on the electrochemical determination of MNZ using modified electrodes [4,12-19]. However, there are limited studies on working electrodes modified with conductive polymers [7,11,20].

Pencil leads of graphite origin are cheap, readily available, and disposable electrode materials used in constructing many electrochemical sensors [21]. The pencil leads are produced by heating graphite and clay mixtures at different rates depending on the hardness and then dipping them in oil and wax to ensure surface smoothness [22].

Features such as high sensitivity, selectivity, stability, repeatability, and low cost are essential in electrochemical sensors. Pencil graphite electrodes (PGE) possess these qualities. PGEs have emerged as preferred electrodes in electrochemical analyses due to their porosity, high electrical conductivity, and large specific surface area. Mass transfer and electron transfer occur quickly on the PGE surface in electrochemical processes. The renewable surface property of PGE provides a high degree of repeatability and allows for easy modification. The essential features distinguishing PGE from other carbon electrodes are that it is affordable, commercially available, disposable, and easy to modify. These properties have led to increased use of PGEs in electrochemical studies [23-28]. Additionally, PGEs have shown sensitivity to determining many electroactive molecules when modified [24].

By evaluating this information, the aim is to prepare nanostructured PPy-modified electrodes on the PGE surface for the electrochemical determination of MNZ and to determine the electroanalytical measurement parameters. In electrode modification using nanomaterials, it is possible to increase the active surface area of the electrodes, accelerate electron transfer kinetics, and enhance sensitivity [23,29,30]. It is planned to benefit from these advantages by synthesizing polypyrrole in nanostructure.

2. EXPERIMENTAL SECTION

2.1. Apparatus

PSTrace 5.9 software-supported potentiostat device (A PalmSens brand, EmStat3+ model) was used to produce and overoxidize the electrodes electrochemically, as well as to perform their characterization and MNZ determination (Figure S1). An ultrasonic bath (Çalışkan brand and LAB.ULT.4010 model) was used to remove oxygen from the solutions. Ultrapure water used in the experiments was obtained from the Stak Pure brand device. pH measurements were taken with a pH meter (Mettler Toledo, Seven Compact S210 model). Scanning electron microscope (SEM) images were obtained using the NanoSEM 650 model (FEI Microscope) device.

2.2. Electrodes, chemicals, and solutions

Pencil leads (Faber Castell brand, 0.70 mm, 2B, and serial number 127722) used as working electrodes were purchased from local stationery stores. A Pt plate (Aldrich 99.9%) measuring 10×10 mm was used as the counter electrode. Ag/AgCl (3 M KCl) reference electrode was used in electrode production and overoxidation processes. For MNZ determinations, an Ag/AgCl (3 M NaCl) reference electrode was used. Figure S2 shows the apparatus to which the working electrodes are attached, the Pt plate counter electrode, and the Ag/AgCl (3 M NaCl) reference electrode. The chemicals used in the study, the companies from which these chemicals were supplied, and their purity percentages are listed in Table S1.

To prepare Britton-Robinson (BR) buffer solution was prepared by slowly adding 0.20 M NaOH to the solution containing 0.04 M H_3BO_3 + 0.040 M CH_3COOH + 0.040 M H_3PO_4. A pH meter was used to measure whether BR buffer reached the desired pH value. Phosphate buffer solution (PBS) at different pH values was prepared according to the recipes available on the liverpool.ac.uk website. For each pH value, the calculated amount of solid NaH_2PO_4 and Na_2HPO_4 was dissolved in the appropriate volume of ultrapure water. To adjust the pH value of this solution, which was mixed with a magnetic stirrer, 0.20 M NaOH was added and then diluted with ultrapure to the required volume.

5 randomly selected Flagly brand antibiotic tablets, each stated to contain 500 mg of MNZ, were ground in a mortar. Then, this powdered mixture's amount of 0.753 g (average tablet mass) was magnetically stirred in 300 mL of ultrapure water for 3 hours. Insoluble solid particles were filtered out using coarse filter paper, and the filtrate was diluted with ultrapure water to a final volume of 500 mL. The resulting solution was used as a stock solution for the real sample experiment.

The procedures performed for the solution in which the real sample determinations were made are as follows. The modified electrode that gave the highest response to MNZ was used in these determinations. This electrode is nanofiber structured polypyrrole produced in 5 cycles and overoxidized in 8 cycles by CV method on PGE (PGE/5cycPPyNF-8cycOx) (see section 3.3). A 0.1 M PBS (pH 8) containing 100 µM MNZ was prepared using the stock solution prepared according to the above procedure. After the measurement was taken with PGE/5cycPPyNF-8cycOx, a standard MNZ stock solution prepared using analytical purity MNZ was added to the determination medium to achieve the total MNZ concentrations 200, 300, and 400 µM.

2.3. Preparation of PGEs for modification

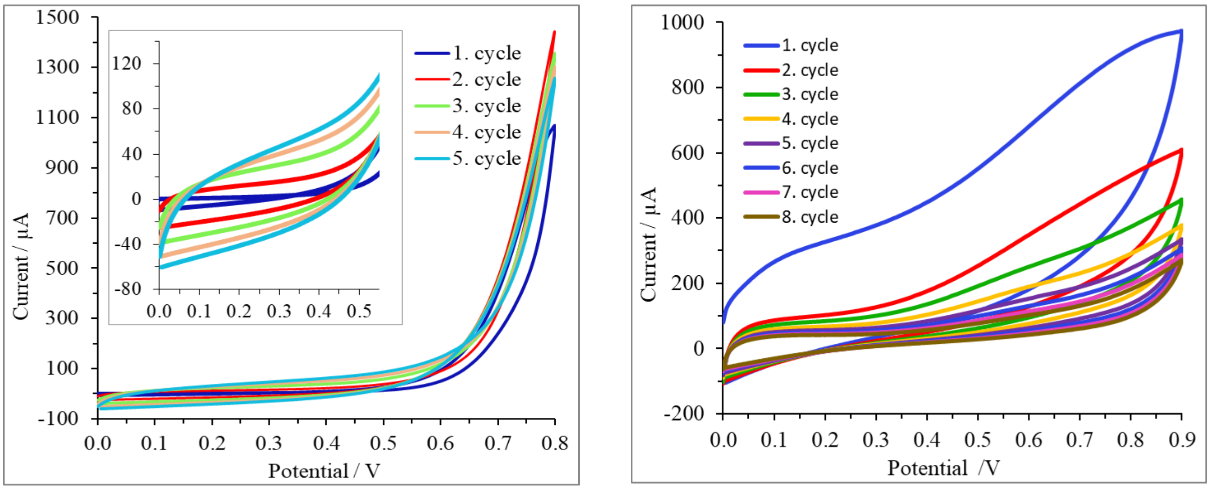
The 6 cm long pencil tips were divided into equal parts, each measuring 3.0 cm, to obtain more PGE. The PGEs were washed ultrasonically for 5 minutes each with water, ethanol, and water again. These washing processes cleaned the PGEs of surface contaminants or tiny carbon particles. Subsequently, the pen tips were dried in an oven at 50°C for 1 hour. The mouths of the beakers containing the dried pencil tips were covered with parafilm to protect them from environmental pollution.

2.4 Modification of PGEs with polypyrrole

Pyrrole monomer was distilled using a rotary evaporator (Heidolph) at 60°C and 130 revolutions per minute to separate it from non-monomer species and stabilisers. Distilled pyrrole was stored at -18 °C in a light-tight glass bottle.

The coating and over-oxidizing of PPy on the surface of PGEs were carried out using the cyclic voltammetry (CV) technique, following the conditions outlined in Özcan et al.'s study [25]. Additional optimizations were made for some parameters as needed in the subsequent stages. A potential range (υ: 100 mV s^{-1}) between 0.0 V and 0.8 V was applied to a 1.20 cm section of the PGEs immersed in a solution containing 100 mM pyrrole, 0.10 M $LiClO_4$, 0.10 M Na_2CO_3. This procedure enabled the formation of nanofiber-structured PPy (PPyNF) on the PGE surface. In electroanalytical measurements, 1.0 cm sections of the modified electrodes are immersed in the measurement solution. The nanofiber structure of PPy on the PGE (PGE/PPyNF) surface is attributed to the presence of Na_2CO_3 in the electropolymerization solution. SEM images obtained in previous studies have also shown that the PPy conductive polymer obtained in this way consists of nanofibers [31]. After PGE/PPyNFs obtained by electropolymerization were washed with deionized water, they were immersed in 10% $HClO_4$ solution for 24 hours to remove the carbonate ions on their surfaces, converting them into carbon dioxide. They were then rewashed with water. PGE/PPy electrodes produced in an environment without Na_2CO_3 were washed only with pure water without being immersed in $HClO_4$.

Pencil graphite electrodes modified with nanofiber structured polypyrrole (PGE/PPyNF), prepared according to the above procedures, were overoxidized by applying eight cycles in the potential range of 0.0 V to 0.90 V (υ: 100 mV s^{-1}) in 0.15 M NaOH solution. Subsequently, the overoxidized electrodes were washed with pure water. Figure 1(a) shows the voltammograms obtained during the formation of nanofiber-structured PPy on the PGE surface by 5-cycle electropolymerization. The electroactivity of the surface changes as the thickness/amount of PPy formed on the PGE surface increases after each cycle, starting from the first cycle. In the first cycle, no significant electroactivity is observed between 0.0 and 0.40 V. When the voltage value exceeds 0.50 V, the increase in current indicates that pyrrole begins to oxidize and polymerization begins. While the oxidation peak voltage value is around 0.75 V, the current value corresponding to this value is over 1.0 mA. Due to the electroactivity of nanofiber-structured PPy on the PGE surface, a current was observed potential range of 0.0 V to 0.50 V in the second and subsequent cycles. This current, which is not observed in the first cycle, reaches higher values as the number of cycles increases.



(a) (b)

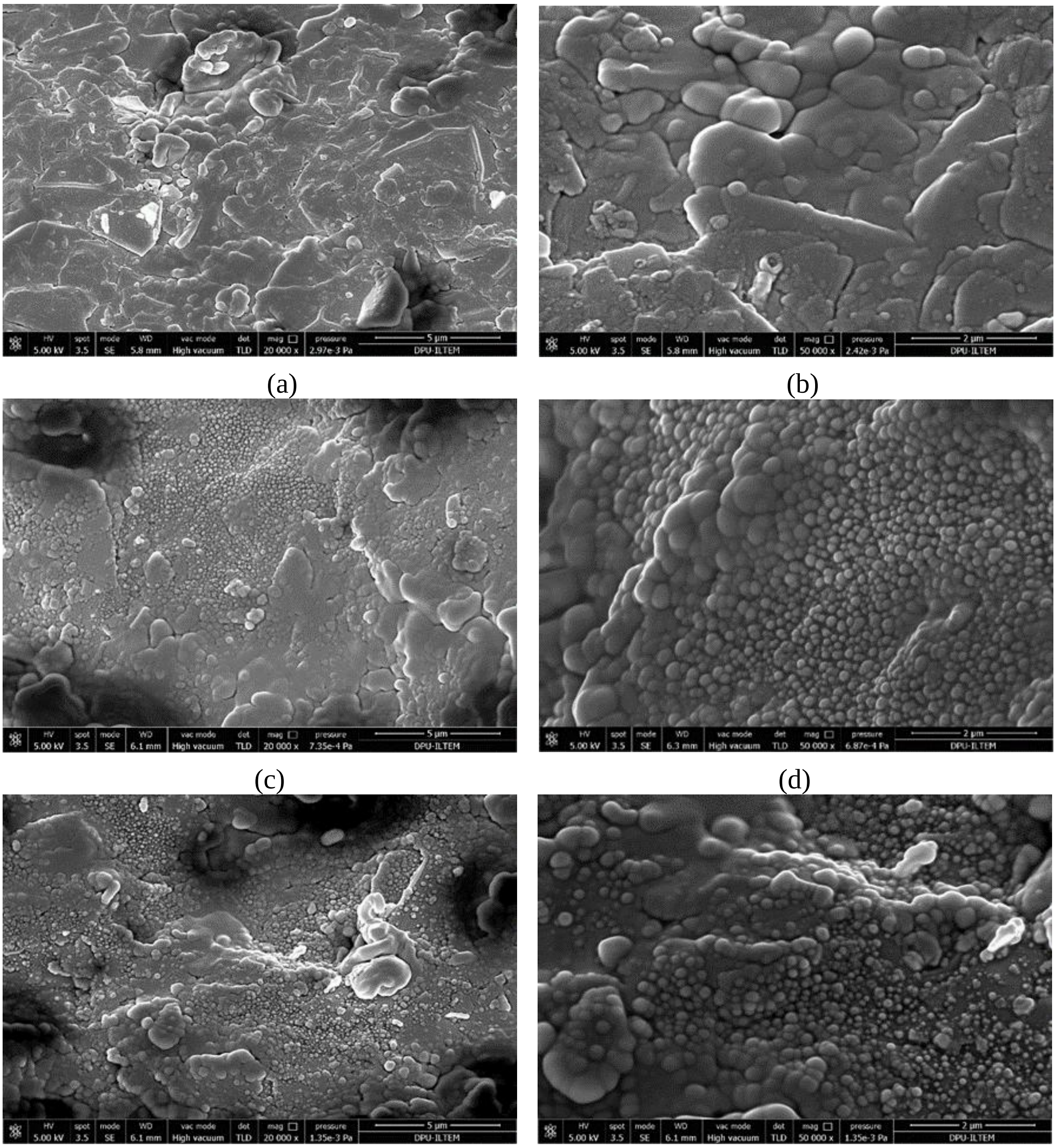
Figure 1. (a) Voltammograms obtained during PPyNF formation on the surface of PGE in 100 mM Py + 0.10 M $LiClO_4$+ 0.10 M Na_2CO_3 solution (υ: 100 mV s^{-1}); b) Voltammograms of overoxidation of PGE/PPyNF in 0.15 M NaOH solution (υ: 100 mV s^{-1})

Figure 1(b) shows the cyclic voltammograms obtained during the overoxidation of PGE/PPyNF in 0.15 M NaOH solution. When PGE/PPyNF is applied for eight cycles between 0.0 V and 0.90 V in NaOH solution, the current value resulting from the electroactivity of PPyNF gradually decreases. This process reduces the electroactivity of PPyNF to levels that do not affect the analyte signals. Especially in the first cycle, the very obvious and high current values around 0.70 V decreased significantly after the fifth cycle. They decreased to levels that showed no significant electroactivity at the end of the 8th cycle.

3. RESULTS AND DISCUSSION

3.1. Surface morphology of polypyrrole-modified electrodes

The surface morphology of PPy structures formed by the electropolymerization method on the PGE surface was examined by SEM. The morphology of PPys is influenced by the presence of ions in the polymerization medium. In the presence of $LiClO_4$ in solution, PPy with a morphology resembling cauliflower is formed on the PGE surface, while PPy with a nanofiber structure is formed in the presence of $LiClO_4$ and Na_2CO_3. Figure 2 shows the SEM images of PPy obtained on the surface of PGE/5cycPPy-8cycOx, PGE/5cycPPyNF, and PGE/5cycPPyNF-8cycOx. Among the SEM images of modified electrodes, those in Figure 2(a-c-e) were acquired with 20000 magnification, while those in Figure 2(b-d-f) were acquired with 50 000 magnification. The 20000 magnification SEM images in Figure 2(a-c-e) provide information about the general appearance of the surface morphology. In the 50000 magnification images in Figure 2(b-d-f), the nanometer scale details of the modified surfaces are seen more clearly.



(a) (b)

(c) (d)

(e) (f)

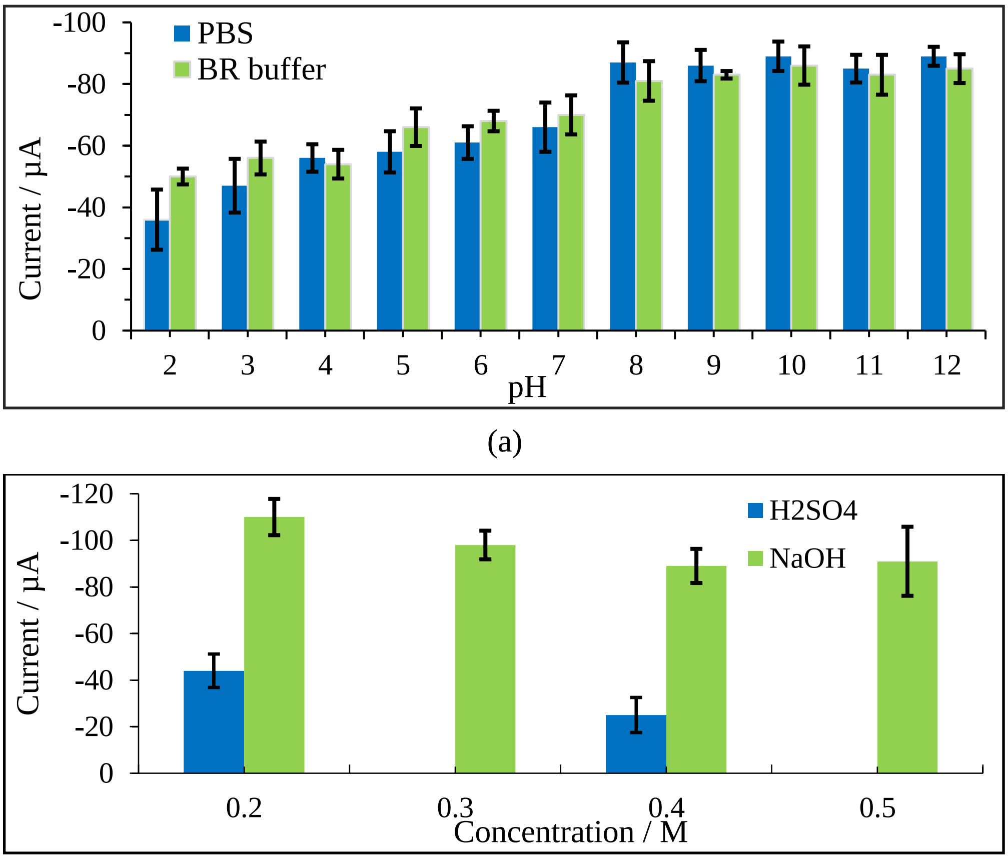
Figure 2. SEM images PGE/5cycPPyNF-8cycOx (a and b), PGE/5cycPPyNF-8cycOx (c and d), and PGE/5cycPPyNF (e and f)

When examining the surface morphology of the PGE/5cycPPy-8cycOx produced under optimum conditions, it resembles a cauliflower structure (Figures 2a and 2b). Upon examining the surface morphology of PGE/5cycPPyNF-8cycOx, it is observed that nanofiber structures begin to form, but they are quite short (Figure 2(c-d) and Figure 2(e-f)]. The shortness of these structures is due to the low number of cycles applied for electropolymerization. It has been shown in the literature that the PPy conductive polymer obtained in a solution similar to the polymerization conditions in this study has a longer nanofiber structure. Nevertheless, it is known that nanofiber structures are obtained by using higher monomer concentrations and the number of cycles [31].

Although the nanofiber structures are short, their surface area increases compared to the PGE/PPy-Ox. When Figure 2(c-d) is compared with Figure 2(e-f) to understand the effect of over-oxidation on the surface morphology, it is seen that over-oxidation does not make a noticeable change in the morphology of the nanofiber structure. However, from electrochemical measurements of MNZ determination, it was determined that excessive oxidation caused an improvement in the signal (see Figure 5).

3.2. Effect of pH on reduction of metronidazole

The pH of the electrochemical determination solution is a crucial parameter affecting the analyte signal. Initially, the optimal conditions outlined in Özcan's study [25] were employed.



(a)

(b)

Figure 3. (a) Peak currents obtained by pre-deposition of PGE/PPyNF-Ox in PBS and BR buffer solutions at different pH values containing 500 µM MNZ (b) Peak currents obtained by pre-deposition of PGE/PPyNF-Ox in NaOH and H_2SO_4 solutions of different concentrations containing 500 µM MNZ (Measurement technique: DPV; Electrode production conditions: 5 cycles, over-oxidation cycles: 15 cycles; deposition conditions; deposition potential -0.5 V and duration 60 s)

PPy was produced in 5 cycles between 0.0-0.80 V in a solution containing 100 mM Py and overoxidized in 15 cycles in 0.15 M NaOH solution. Determination of MNZ using the differential pulse voltammetry (DPV) technique was carried out by accumulation (60 s at -0.50 V). These parameters were later re-optimized again in subsequent stages.

In this study, PBS and BR buffer solutions, commonly utilized in electrochemical investigations, were examined. Measurements were performed using the DPV technique with pre-deposition in 0.10 M PBS and 0.040 M BR buffer solutions at various pH values, each containing 500 µM MNZ. The data presented in Figure 3 indicates that the MNZ reduction peak currents in the PBS are higher than those in the BR buffer solution.

Additionally, it is observed that the reduction peak current of MNZ is higher in the basic solution. For this reason, the reduction current of MNZ was also determined in more basic and more acidic solutions. Figure 3(b) illustrates the electrochemical measurement outcomes in different concentrations of NaOH and H_2SO_4 solutions, both containing 500 µM MNZ. The results presented in Figure 3(a) and (b) show that the highest reduction peak current for MNZ was achieved in a 0.20 M NaOH solution. However, the current value in 0.20 M NaOH solution is not sufficiently high to be preferred over those in buffer solution environments. Taking into account the pH values of human body fluids, the optimum pH and solution environment were evaluated to be 0.10 M FT at pH 8.0. Therefore, subsequent MNZ determinations were carried out in this solution.

3.3. Determination of optimum conditions for electrode modification

Since the parameters related to the preparation and over-oxidation of nanostructured PPy can influence the electrochemical signals, optimization studies have been conducted for them. The optimization of PGE/PPyNF-Ox used in MNZ determination was assessed in two stages. Initially, the parameters determining the thickness of PPyNF formed on the PGE surface were examined. Factors influencing the PPyNF film thickness and nanofiber length formed on the PGE surface include the number of cycles and Py concentration used in polymer synthesis. Following investigations into these parameters, the second stage focused on studying the effects of over-oxidation process parameters aimed at reducing the electroactivity of PPyNF in MNZ determination.

3.3.1. Determination of Number of Cycles

PGE/PPyNF-Ox was synthesized by applying CV for 1-12 cycles in the polymerization solution containing 100 mM Py. DPV measurements were performed using these electrodes in the PBS at pH 8.0 containing 500 µM MNZ. The effect of the number of cycles applied to fabricate modified electrodes on the MNZ reduction peak current is depicted in Figure 4. Additionally, for comparative purposes, the results for PGE and PGE/Ox are also presented in Figure 4. When Figure 4 is examined, it is seen that the reduction peak currents of MNZ increase by modifying PGE with PPy. The current value rises to 5 cycles, beyond which no significant increase is observed, and there is a decline in the current value after 12 cycles. Consequently, the number of cycles required for producing PGE/PPyNF was determined as 5. In subsequent optimization studies, PGE/PPyNF was produced in 5 cycles. The abbreviation for these electrodes is PGE/5cycPPyNF.

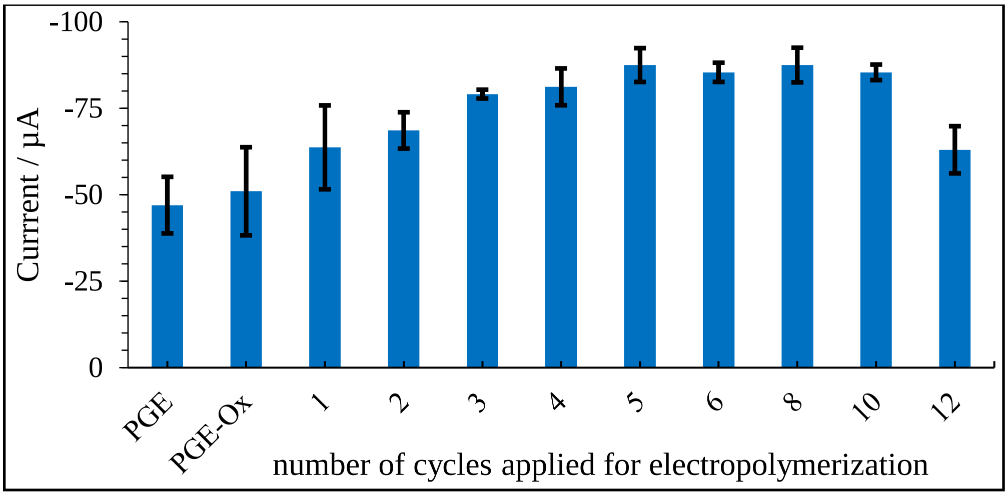


Figure 4. Effect of the number of cycles applied as CV in obtaining PGE/PPyNF-Ox on the reduction peak current of 500 µM MNZ (PBS at pH 8.0) (Number of over-oxidation cycles: 15 (in 0.150 M NaOH), deposition potential: -0.50 V, deposition time 60 s)

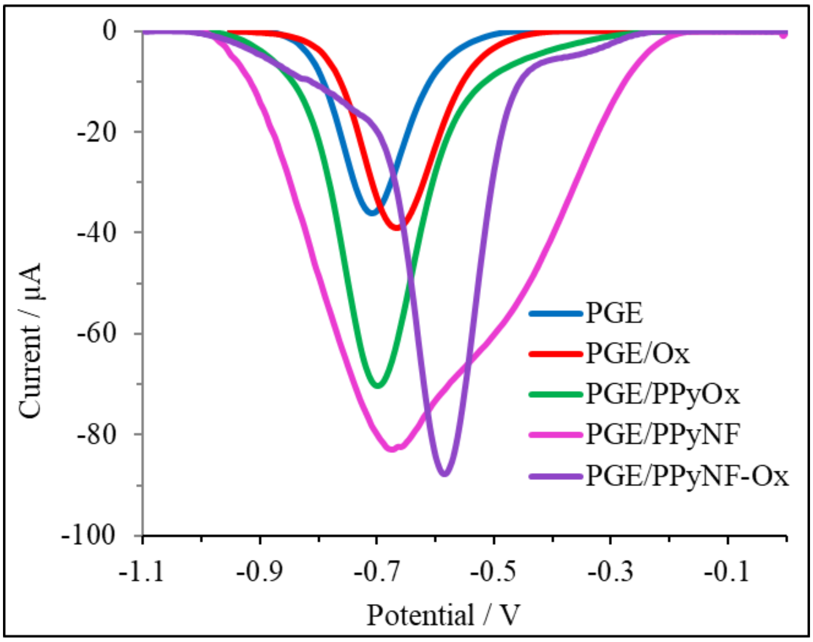


Figure 5. DP voltammograms of PGE, PGE/Ox, and PPy-modified PGE in PBS at pH 8.0 containing 500 µM MNZ (number of oxidation cycles: 15, deposition voltage: -0.50 V, deposition time: 60)

Figure 5 displays the voltammograms acquired through DPV for 500 µM MNZ (in PBS at pH 8.0) using PGE, PGE/Ox, PGE/5cycPPyNF, PGE/5cycPPy-Ox, and PGE/5cycPPyNF-Ox. The nanofiber structure of the modified PGE surface confers a significant advantage in the determination of MNZ. As depicted in Figure 5, the nanofiber structure of the electrode surface enhances the peak reduction current of MNZ. In other studies reported in the literature, an enhancement in the electrochemical signal of analytes was observed with the over-oxidation of PPy. This signal increase was attributed to the increase of -COO- and -COOH groups on the PPy surface [32]. Furthermore, modification of PGE with PPyNF and over-oxidation increased the MNZ reduction peak current. In addition, if PGE is solely modified with PPyNF, no peak is observed. These findings indicate that utilizing PGE/5cycPyNF-Ox as the working electrode is appropriate.

3.3.2. Determination of Pyrrole Concentration

Another factor influencing the thickness of the PPy film modified on the PGE surface is the concentration of Py monomer in the electropolymerization solution. The following procedures were performed to determine the effect of Py concentration. PGE/5cycPPyNF was synthesized using CV in the 0.0-0.80 V range in the electropolymerization solution containing Py at concentrations between 10-120 mM.

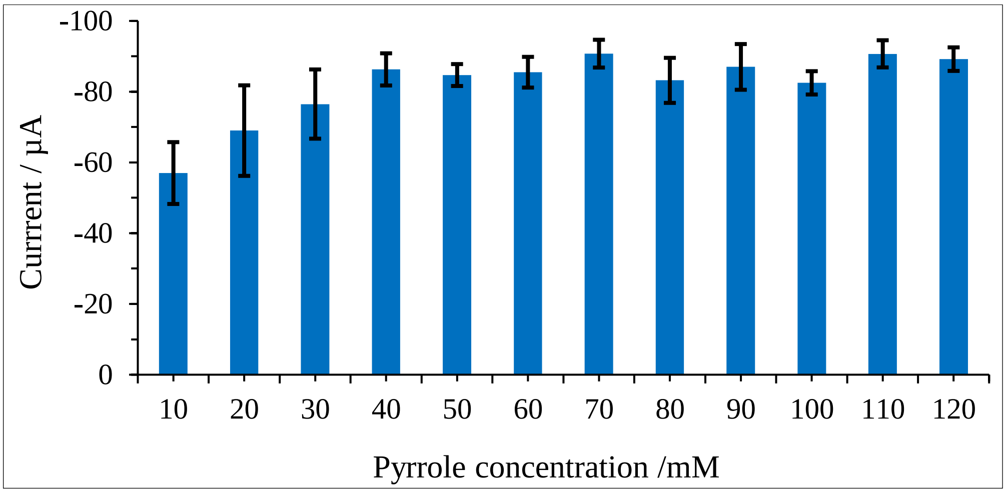


Figure 6. The effect of the Py concentration used in the preparation of PGE/5cycPPyNF-Ox on the reduction peak current obtained for 500 µM MNZ (in PB solution at pH 8.0) (Number of electrode over-oxidation cycles: 15, deposition voltage: -0.50 V, deposition time: 60 s).

Then, these electrodes were over-oxidized in 0.15 M NaOH solution with CV in the voltage range of 0.0-0.90 V for 15 cycles. These electrodes are denoted as PGE/5cycPPyNF-Ox. DPV measurements were conducted in the PBS at pH 8.0 containing 500 µM MNZ using PGE/5cycPPyNF-Ox produced in the solution containing Py at different concentrations (Figure

6). When Figure 6 is examined, it can be seen that the highest peak current value, -90.75 µA, was reached with the PGE/5cycPPyNF-Ox produced in a solution containing 70 mM Py. However, the MNZ reduction peak current values of electrodes obtained from solutions containing Py between 40-120 mM are very close. Among these values where the same results can be obtained, 40 mM Py concentration, which requires less monomer consumption, was chosen.

3.4. Determination of the number of cycles used for over-oxidation

Preparation of the modified electrodes for MNZ determination takes place in two stages. The first stage is the modification of the PGE, and the second stage is the overoxidation of the modified electrodes. After the modification parameters for PGE/PPyNF-Ox are determined, the parameters of the over-oxidation process applied to prevent electroactivity arising from PPyNF in the determination of MNZ should be determined. In the part of this work so far, following the methodology outlined by Özcan [25], the overoxidation of the PGE/PPyNF was carried out in 0.15 M NaOH solution for 15 cycles using CV within the voltage range of 0.0-0.90 V (υ: 100 mV s^{-1}). Additionally, in this study, the number of cycles applied in the over-oxidation of the PGE/5cycPPyNF was optimized for MNZ determination (Figure S3). The data in Figure 5 and Figure S3 demonstrate that over-oxidation of the PGE/5cycPPyNF electrode affects the reduction current obtained for MNZ. Examining Figure S3, the highest peak current value of -88.3 µA was reached with the over-oxidized electrode in 13 cycles. However, there is no significant difference in the peak currents obtained with over-oxidized electrodes with 8 (-87.5 µA) or more cycles. Since practicality and rapidity are also important parameters in MNZ determination, it was decided that the number of over-oxidation cycles should be 8. In subsequent studies, PGE/PPyNF were over-oxidized in 0.15 M NaOH solution using the CV technique in the potential range of 0.0-0.90 V (100 mV s^{-1}) for 8 cycles. The electrodes produced in this manner are abbreviated named PGE/5cycPPyNF-8cycOx.

3.5. Effect of pre-deposition on metronidazole reduction peak current

In this section, the effect of deposition parameters on MNZ reduction was examined. Measurements were carried out using PGE/5cycPPyNF-8cycOx in PBS at pH 8.0 containing 500 µM MNZ, with and without accumulation, by the DPV method. The results obtained with DPV at different deposition voltages for a deposition time of 60 seconds and without deposition are shown comparatively in Figure S4. The highest peak current was recorded when the deposition was applied at the voltage of -0.30 V. This current value is the same as the current value obtained without accumulation (-92.6 µA). In addition, when the voltammograms are examined, the reduction peak current values and voltage values are close. For this reason, it was decided that MNZ determination could be made with DPV without accumulation in subsequent measurements.

3.6. Dependence of the electrochemical response of metronidazole on concentration

DPV measurements were conducted in solutions containing MNZ at concentrations ranging from 5 to 2500 µM to ascertain the linear working range, The variation in the peak reduction current obtained by DPV in 0.10 M PBS at pH 8.0 containing different MNZ concentrations with PGE/5cycPPyNF-8cycOx is given in Figure 7. The linear working range for PGE/5cycPPyNF-8cycOx was determined as 5-500 µM (R^2 =0.9985). The differential pulse voltammograms obtained for concentrations within the linear range are seen in Figure 8. No significant peak could be observed in the DP voltammograms obtained in solutions containing MNZ at concentrations lower than 5 µM. The limit of detection (LOD) calculated for MNZ determinations using PGE/5cycPPyNF-8cycOx is 3.8 µA, and the limit of quantification (LOQ) is 12.8 µA.

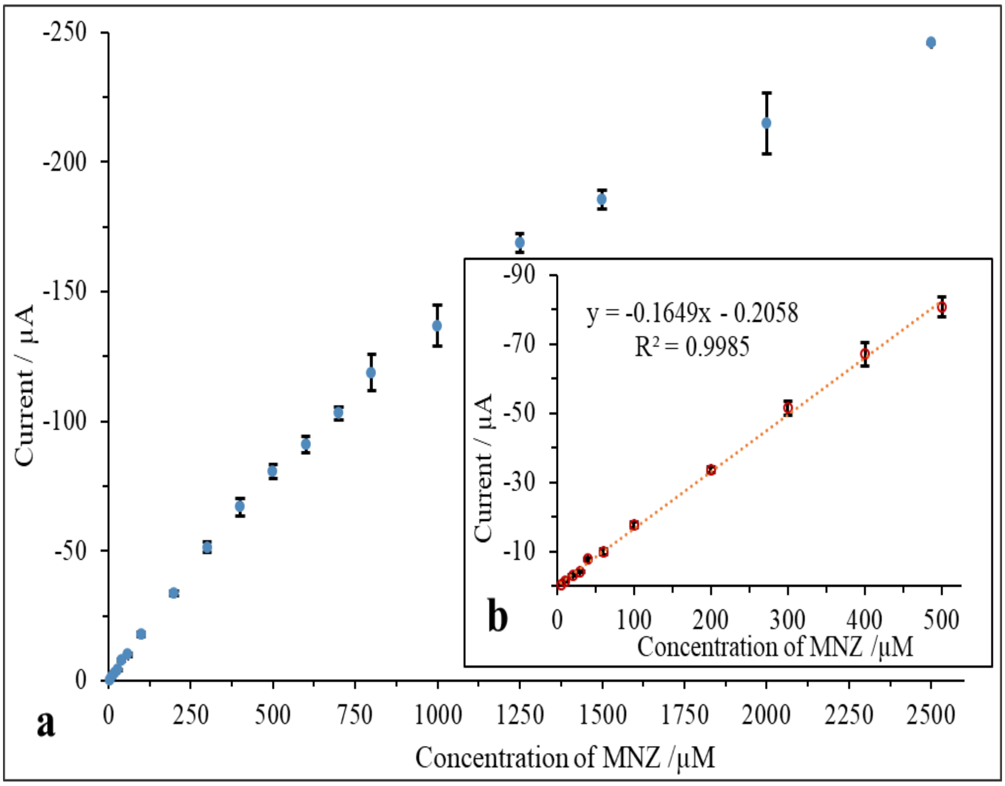


Figure 7. (a) Graph of the current values obtained in PBS at pH 8.0 containing different MNZ concentrations by the DPV method with PGE/5cycPPyNF-8cycOx, (b) Linear range obtained for MNZ with PGE/5cycPPyNF-8cycOx

3.7. Electrochemical characteristics of PPy-modified electrodes

The cyclic voltammograms obtained for PGE, PGE/Ox, PGE/5cycPPy-8cycOx, and PGE/5cycPPyNF-8cycOx in a solution containing 0.10 M KCl + 15 mM pH 7.4 PBS + 5.0 mM $K_3Fe(CN)_6$ and $K_4Fe(CN)_6$ are presented in Figure S5. ΔEp and anodic peak current values obtained using these voltammograms are shown in Table 1. When the data obtained is evaluated, it is understood that the modifications made alter the surface characteristics of PGE.

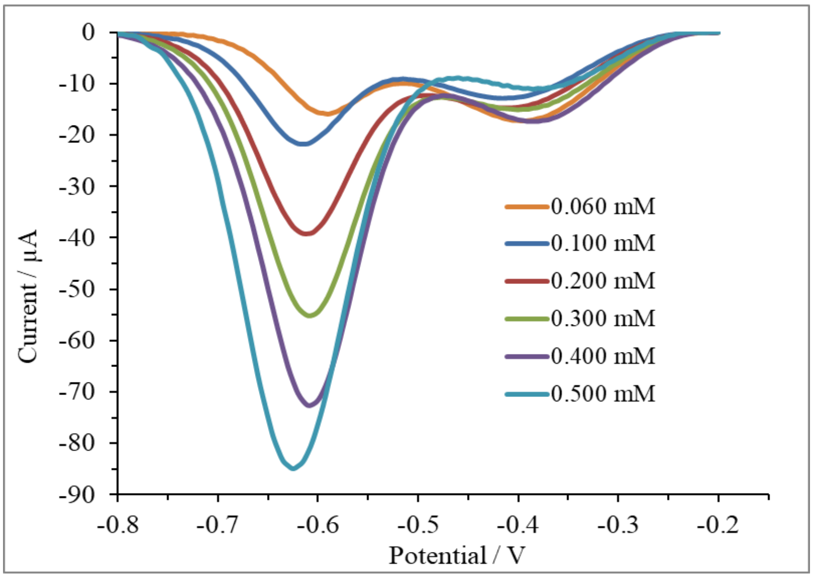


Figure 8. DP voltammograms obtained for MNZ in the linear range within PBS at pH 8.0 using the PGE/5cycPPyNF-8cycOx

Cyclic voltammograms obtained for scan rates ranging from 10 to 400 mV s^{-1} for PBS at pH 8.0 containing 500 µM MNZ using PGE/5cycPPyNF-8cycOx and the change of current values with scan rate are presented in Figure 9. The graph of the square root of the scan rate versus the reduction peak current is seen in Figure S6(a), and the graph of the logarithm of the scan rate versus the logarithm of the peak current is seen in Figure S6(b). The fact that the scan rate-peak current graph is non-linear, the graph in Figure S6(a) is linear, and the slope value of the graph in Figure S6(b) is close to 0.5 indicates that the process is diffusion-controlled.

Table 1. Peak-peak separation (ΔEp) and anodic peak current values of redox peak pairs for PGE, PGE/Ox, PGE/5cycPPy-8cycOx, and PGE/5cycPPyNF-8cycOx

Electrode	**E$_{\text{Anodic Peak}}$ (mV)**	**E$_{\text{Cathodic Peak}}$ (mV)**	**ΔEp (mV)**	**I$_{\text{Anodic peak current}}$ (µA)**
PGE	260	145	115	473
PGE/8cycOx	365	35	300	169
PGE/5cycPPy-8cycOx	325	60	265	103
PGE/5cycPPyNF-8cycOx	285	105	180	237

In addition, calculations were made using the data obtained in the pH study (Section 3.2) to determine the electrochemical reduction mechanism of MNZ. Figure S7(a) shows the variation in the reduction peak voltages of MNZ in solutions containing 500 µM MNZ at different pH values. The slope of the line obtained for pH values between 2.0-8.0 in Figure S7(b) is close to 59 mV (62.3 mV), providing data that the number of electrons and protons transferred in the MNZ reduction reaction is equal. Moreover, according to the study of Laviron [33], for irreversible systems, the following equation relates the peak voltage (E_p) to the natural logarithm of the scan rate (ln ν), where the slope of the Ep-ln ν line is equal to RT/αnF.

$$Ep = E0' + \frac{RT}{anF} ln\nu$$

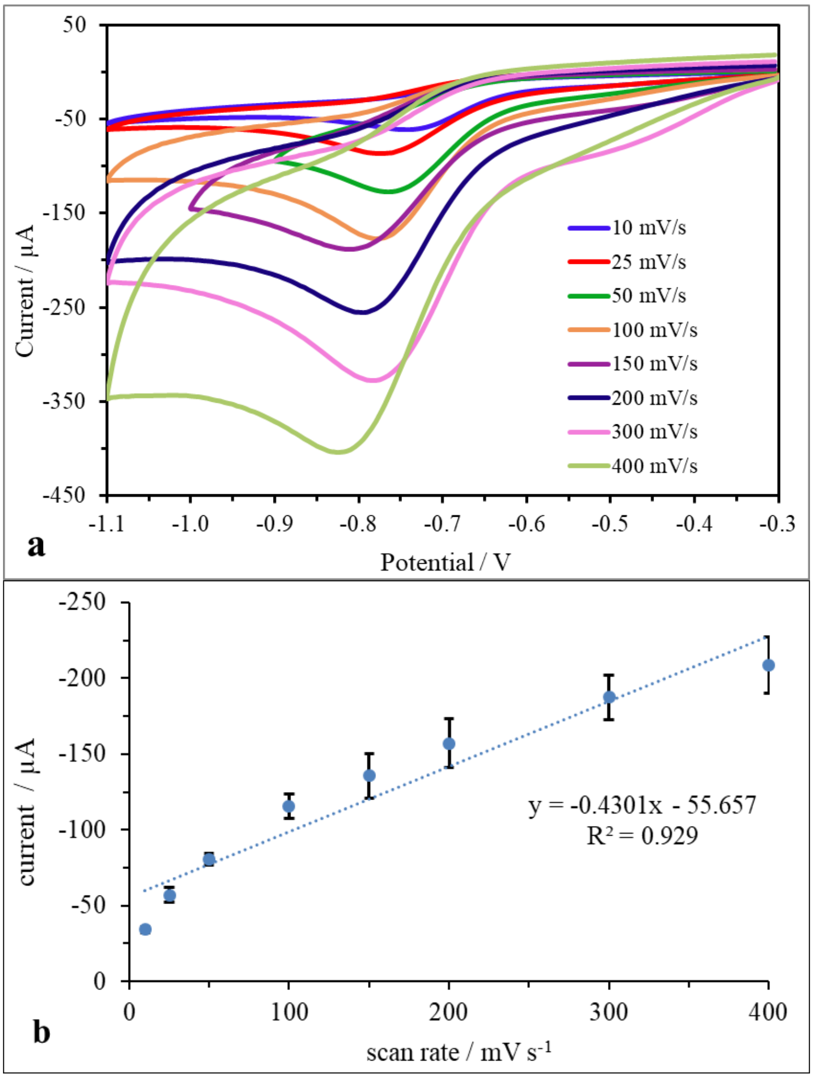


Figure 9. (a) Cyclic voltammograms obtained at different scan rates in PBS at pH 8.0 containing 500 µM MNZ using PGE/5cycPPyNF-8cycOx; (b) Peak current-scan rate graph

To calculate the n value, the slope of the line in Figure S7(c) was used, resulting in an αn value of 1.87, and the number of electrons transferred was found to be 3.75 (about 4). Using these data, the mechanism for the reduction of MNZ is proposed as shown in Figure 10. This mechanism has been suggested in some studies where MNZ was determined with modified electrodes [34].

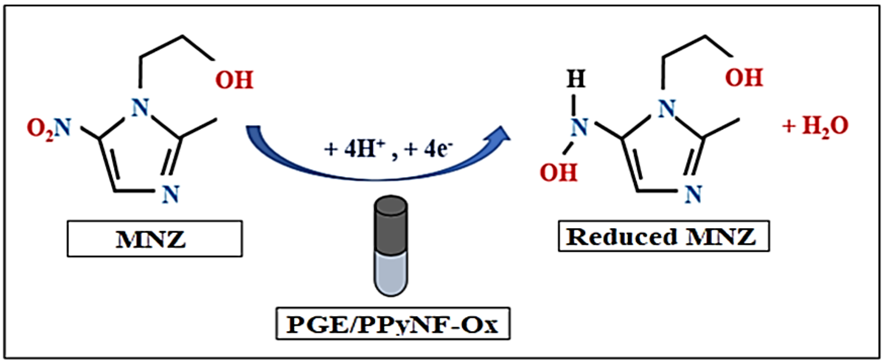


Figure 10. Reduction mechanism of MNZ by PGE/5cycPPyNF-8cycOx at PBS at pH 8

Table 2. Effect of interferent species on the peak reduction current obtained by the DPV method for 100 µM MNZ with PGE/5cycPPyNF-8cycOx

Interferent species	**Concentration of interferent species (µM)**	**Change in current obtained with PGE/5cycPPyNF-8cycOx for 100 µM MNZ[a] (µA)**	**Deviation in current (%)**
Ascorbic acid	50	+0.06	0.36
	100	+0.36	2.18
	250	+0.32	1.92
	500	+1.18	4.93
Glucose	100	+0.03	0.15
	250	+0.28	1.67
	500	+0.22	1.33
	750	+0.33	1.97
KCl	50	-0.18	1.13
	100	-0.24	1.45
	250	-0.31	1.85
	500	-0.25	1.52
NaCl	50	+0.21	1.27
	100	+0.44	2.66
	250	+0.82	4.90
	500	+0.86	5.12
Paracetamol	50	+0.04	0.25
	100	+0.28	1.71
	250	+0.37	2.23
	500	+0.46	2.73

[a] Using the calibration curve, the current value obtained for PGE/5cycPPyNF-8cycOx is -16.71 µA

3.8. Effect of interfering species on the reduction of metronidazole

Since there are many molecules in body fluids and wastewater, it is important to determine how the presence of various molecules in the environment affects the determination of MNZ. DPV measurements were conducted using PGE/5cycPPyNF-8cycOx in PBS at pH 8.0 containing 100 µM MNZ for different concentrations of interferent species. The effect of these species on the electrochemical MNZ determination was determined. The change in MNZ reduction peak currents obtained in the presence of interferent species and the deviation percentages of the change are presented in Table 2.

When Table 2 is examined, no significant change is observed in the MNZ reduction peak current under conditions where the ascorbic acid concentration is 2.50 times the MNZ concentration and the paracetamol concentration is 5 times the MNZ concentration. In the presence of ascorbic acid 5 times the MNZ concentration, the difference in the PGE/5cycPPyNF-8cycOx response approached 5% (4.93%). The fact that ascorbic acid is an electroactive molecule explains this change in the signal. In the MNZ determination medium, the presence of glucose up to 7.5 times the MNZ concentration changes the current value in MNZ determination by a maximum of 2%. The presence of NaCl in the determination medium affected MNZ determinations more than the presence of KCl. However, the effect of these salts on the MNZ current remains below 5%.

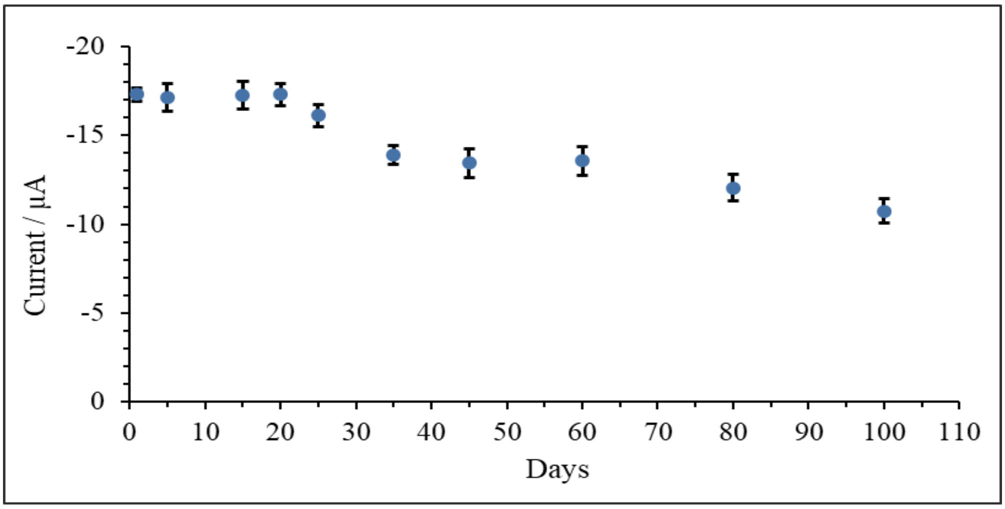


Figure 11. Measurement results obtained on different days with DPV for 100 µM MNZ with PGE/5cycPPyNF-8cycOx

3.9. Lifetime and reproducibility of PGE/5cycPPyNF-8cycOx

To determine the change in the electrochemical responses of PGE/5cycPPyNF-8cycOx over time, a large number of electrodes were produced and overoxidized on the same day. When not in use, these electrodes were stored in a vacuum desiccator at room temperature. Measurements were performed on different days using PGE/5cycPPyNF-8cycOx with the

DPV technique in 0.10 M PBS at pH 8.0 solution containing 100 µM MNZ. The average of the current values obtained as a result of 6 measurements performed under the same conditions with disposable PGE/5cycPPyNF-8cycOx for different days is shown in Figure 11. A decrease in the current became noticeable after the 20th day. By the 100th day, the MNZ reduction peak current value had decreased by 38% compared to the value recorded on the first day.

The reproducibility of PGE/5cycPPyNF-8cycOx was assessed by calculating the relative standard deviation of MNZ reduction peak current values, resulting in 5.87%. This value was obtained from DPV measurements conducted with six electrodes fabricated separately in a PBS at pH 8.0 containing 100 µM MNZ. The relatively low relative standard deviation indicates the surface stability and repeatability of disposable PGE/5cycPPyNF-8cycOx.

3.10. Measurements in pharmaceutical tablets containing metronidazole

The preparation of a stock solution from a drug sample to determine the real sample (drug) is explained in Section 2.2. The concentration of the real sample containing 100 µM MNZ (drug) was calculated as 99.4 µM by the standard addition method using PGE/5cycPPyNF-8cycOx electrodes. Figure S8 illustrates the graph generated from the study data. This result demonstrates that the PGE/5cycPPyNF-8cycOx produced in this study can effectively determine the amount of MNZ in real samples such as pharmaceutical tablets.

4. CONCLUSION

Nanostructured PPy conductive polymer, which can be used in MNZ determinations, was produced by cyclic voltammetry on the PGE surface. Nanostructured electrodes were overoxidized electrochemically to ensure that their electroactivity did not interfere with the analyte response. This overoxidation also improved the signal of the modified electrodes. Optimization studies have been carried out for the parameters affecting MNZ determinations. The optimizations revealed that for MNZ determinations, the optimal Py concentration in PGE/PPyNF-Ox production was 40 mM, and the ideal number of cycles was 5. It was also determined that 8 cycles were adequate for over-oxidation. Subsequent experimentation showed that pre-deposition with the electrode produced under optimum conditions (PGE/5cycPPyNF-8cycOx) did not enhance the reduction current of MNZ. For PGE/5cycPPyNF-8cycOx, MNZ reduction was found to be predominantly diffusion-controlled. In addition, electrochemical data calculations demonstrated that MNZ reduction occurred with the transfer of 4 electrons and 4 protons. In measurements made with DPV in the presence of MNZ, the electrochemical response of PGE/5cycPPyNF-8cycOx was found to be linear between 5 µM and 500 µM, and the LOD value was calculated as 3.8 µM and the LOQ value was 12.8 µM. The presence of interferent species did not significantly affect the results of MNZ determination. The reproducibility, as indicated by the relative standard deviation of measurements conducted with 6 different electrodes, was 5.87%. The signal of modified electrodes remained stable for 20 days, and successful determination of MNZ in pharmaceutical tablets was demonstrated.

Table 3. Comparison of the electroanalytical characteristics of PGE/5cycPPyNF-8cycOx in MNZ determinations with the literature

Modified Electrode	Linear range (μM)	LOD (μM)	Reference
Gr-IL-GCE[a]	0.1–25	0.047	[12]
β -CD-GNPs-poli(L -cys)-GCE[b]	0.1-600	0.014	[13]
DMIPS-CPE[c]	0.4-200	0.01	[35]
Polydopamine-MWCNTs–COOH-GCE[d]	5-5000	0.25	[15]
CS-PC BPE-GCE[e]	0.01-465	0.009	[16]
PARS-GCE[f]	0-125	0.38	[18]
AgNP-CuMOF-PPy–rGO-CPE[g]	0.08-160	0.024	[20]
PGE/PPyNF-Ox[h]	5-500	3.8	This study

[a] Graphene-Ionic liquid-glassy carbon electrode
[b] β -cyclodextrin-functionalized gold nanoparticles-Poly(L-cysteine)-glassy carbon electrode.
[c] Dublex molecularly imprinted polymer- glassy carbon electrode
[d] Polydopamine –Multiwall Carbon nanotube-carboxylic- glassy carbon electrode
[e] Chitosan-pectin bio polyelectrolyte- glassy carbon electrode
[f] Poly(alizarin red s)-glassy carbon electrode
[g] Silver nanoparticles- Metal organic framework-reduced graphene oxide coated polypyrrole-carbon paste electrode
[h] Pencil graphite electrode-over oxidized polypyrrole nanofiber

To enable comparison with the literature, the linear range and LOD of the electrodes developed for MNZ determination in previous studies can be seen in Table 3. Although the LOD values are at higher concentrations in this study than in other studies, the linear working range is wider than in most studies. With a simple modification, cheap and disposable nanostructured electrodes that can be used effectively in MNZ determinations in real samples have been produced and used successfully.

Declarations of interest

The authors declare no conflict of interest in this reported work.

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